6. New active ribosomes (heavier than the 80s monomers) appeared to be formed and the synthesis of haemoglobin was increased when RNA was added to the cell-free system containing light ribosomes.

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REFERENCES

Allen, E. H. & Schweet, R. S. (1962). *J. biol. Chem.* **237**, 760.

Arlinghaus, R. & Schweet, R. (1962). *Biochem. biophys. Res. Commun.* **9**, 482.

Arnstein, H. R. V. & Cox, R. A. (1963). *Biochem. J.* **88**, 27P.

Arnstein, H. R. V., Cox, R. A. & Hunt, J. A. (1962*a*), *Nature, Lond.*, **194**, 1042.

Arnstein, H. R. V., Cox, R. A. & Hunt, J. A. (1962*b*). *Biochem. J.* **84**, 91P.

Barondes, S. H., Dingman, C. W. & Sporn, M. B. (1962). *Nature, Lond.*, **196**, 145.

Bishop, J., Favelukes, G., Schweet, R. & Russell, E. (1961). *Nature, Lond.*, **191**, 1365.

Borsook, H., Fischer, E. H. & Keighley, G. (1957). *J. biol. Chem.* **229**, 1059.

Brenner, S., Jacob, F. & Meselson, M. (1961). *Nature, Lond.*, **190**, 576.

Burt, N. S., Murray, R. G. E. & Rossiter, R. J. (1951). *Blood*, **6**, 906.

Cox, R. A. & Arnstein, H. R. V. (1963). *Biochem. J.* **89**, 574.

Dintzis, H. M. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 247.

Gierer, A. (1963). *J. molec. Biol.* **6**, 148.

Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Risebrough, R. W. & Watson, J. D. (1961). *Nature, Lond.*, **190**, 581.

Hayashi, M. & Spiegelman, S. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 1564.

Hiatt, H. H. (1962). *J. molec. Biol.* **5**, 217.

Holloway, B. W. & Ripley, S. H. (1952). *J. biol. Chem.* **196**, 701.

Huisman, T. H. J., Martis, E. A. & Dozy, A. (1958). *J. Lab. clin. Med.* **52**, 312.

Ishihama, A., Mizuno, N., Takai, M., Otaka, E. & Osawa, S. (1962). *J. molec. Biol.* **5**, 251.

Kruh, J., Dreyfus, J. C. & Schapira, G. (1962). *Biochem. J.* **84**, 91P.

Kruh, J., Rosa, J., Dreyfus, J. C. & Schapira, G. (1961). *Biochim. biophys. Acta*, **49**, 509.

Lamfrom, H. (1961). *J. molec. Biol.* **3**, 241.

Lengyel, P., Speyer, J. F. & Ochoa, S. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 1936.

Mans, R. J. & Novelli, G. D. (1961). *Arch. Biochem. Biophys.* **94**, 48.

Marks, P. A., Willson, C., Kruh, J. & Gros, F. (1962). *Biochem. biophys. Res. Commun.* **8**, 9.

Maxwell, E. S. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1639.

Monier, R., Naono, S., Hayes, D., Hayes, F. & Gros, F. (1962). *J. molec. Biol.* **5**, 311.

Munro, A. J. & Korner, A. (1963). *Nature, Lond.*, **198**, 891.

Nathans, D., Notani, G., Schwartz, J. H. & Zinder, N. D. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1424.

Nirenberg, M. W. & Matthaei, J. H. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 1588.

Nomura, M., Hall, B. D. & Spiegelman, S. (1962). *J. molec. Biol.* **2**, 306.

Ohtaka, Y. & Spiegelman, S. (1963). *Science*, **142**, 493.

Peterson, E. A. & Sober, H. A. (1951). *J. Amer. chem. Soc.* **78**, 751.

Schweet, R. S., Lamfrom, H. & Allen, E. H. (1958). *Proc. nat. Acad. Sci., Wash.*, **44**, 1029.

Ts'o, P. O. P. & Vinograd, J. (1961). *Biochim. biophys. Acta*, **49**, 113.

Tsugita, A., Fraenkel-Conrat, H., Nirenberg, M. W. & Matthaei, J. H. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 846.

Volkin, E. & Astrachan, L. (1956). *Virology*, **2**, 149.

Volkin, E., Astrachan, L. & Countryman, J. L. (1956). *Virology*, **6**, 545.

Warburg, O. & Christian, W. (1942). *Biochem. Z.* **310**, 384.

Warner, J. R., Rich, A. & Hall, C. E. (1962). *Science*, **138**, 1399.

Weinstein, I. B. & Schechter, A. N. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1686.

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Transamination of Peptides

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Despite the considerable literature on non-enzymic transamination of amino acids that has accumulated since the original observations (e.g. Nakada & Weinhouse, 1953; Metzler, Olivard & Snell, 1954*a*; Fleming & Crosbie, 1960), little work has been reported on the application of this reaction to peptides. Dixon & Weitkamp (1962) produced a peptide (Gly[1]-corticotrophin) from the corresponding α-oxo acyl compound; the reverse of this reaction, i.e. the conversion of the *N*-terminal residue of a peptide into an α-oxo acyl residue, is now reported. Cennamo, Carafoli & Bonetti (1956) described the same reaction with pyridoxal as amino acceptor and alum as a catalyst. It is

possible to use mild conditions that might enhance the specificity of the method if use is made of the discovery that pyridine greatly accelerates Cu^{2+} ion-catalysed transaminations (Mix & Wilcke, 1960; Mix, 1961*a*, *b*). The possible use of transamination for the removal of the *N*-terminal residue of a peptide has been indicated in a preliminary account of this work (Dixon, 1964).

METHODS

Chromatography of oxo acids. After the finding that glyoxylic acid failed to give a sharp peak with a column of a weakly basic resin (De-Acidite G SRA 101), possibly owing to the formation of Schiff bases, the material was run on the acetate of a strongly basic resin (De-Acidite FF SRA 71; polystyrene beads containing quarternary ammonium groups; 100–200 mesh; 7–9% cross-linking). After the resin had been washed with concentrated acetate buffer until free from chloride, columns were packed and equilibrated with a buffer containing acetic acid (0·5M), sodium acetate (0·5M) and toluene (0·03%). This system proved suitable (Fig. 1) for isolating pyruvoylglycine (R_F 0·26) from glyoxylate (R_F 0·17) and pyruvate (R_F 0·11). The transamination product from glycylalanine, believed to be glyoxyloylalanine, was at first just separated from glyoxylate by using the above system. In later experiments, possibly with a different batch of resin, neither it nor the product from glycylglycine was separated from glyoxylate. A different preparation of resin of the same general type (De-Acidite FF SRA 72; 200 mesh), with a buffer of double the previous concentration, was successful. In this sytem the R_F values of the presumed glyoxyloylglycine and glyoxylate were 0·20 and 0·14 respectively.

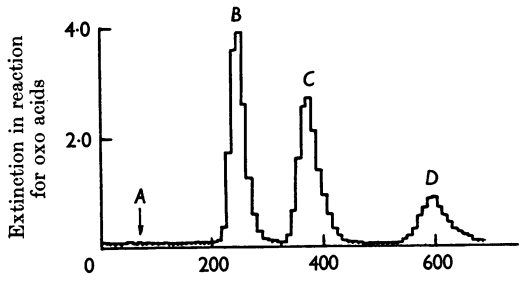


Fig. 1. Chromatography of oxo acids: 3·5 mg. of pyruvoylglycine, 2·4 mg. of glyoxylic acid hydrate and 2·0 mg. of crude sodium pyruvate were dissolved in 0·2 ml. of 0·25M-cupric acetate in 0·1M-acetic acid and 0·8 ml. of eluent. This sample was applied to a column (77 cm. × 1·1 cm.) of the basic resin De-Acidite FF SRA 71, equilibrated and developed with a solution of sodium acetate (0·5M), acetic acid (0·5M) and toluene (0·03%). Samples (1 ml.) were used for the colour reaction with dinitrophenylhydrazine. Other conditions were as described in the text. *A*, Cu^{2+} ions; *B*, pyruvoylglycine; *C*, glyoxylic acid; *D*, pyruvic acid.

Estimation of oxo acids. The method of Lu (1939) as modified by Friedemann & Haugen (1943) was modified somewhat further. The sample was diluted to 1 ml. with water and mixed with 1 ml. of a solution of 0·1% 2,4-dinitrophenylhydrazine in approx. 2N-HCl dissolved by the method of Friedemann & Haugen (1943). After 10 min. or more, 4 ml. of ethyl acetate was added and the phases were mixed for 20 sec. by a stream of air. The ethyl acetate extracted the unexpended reagent less completely than the hydrazones formed. Most of the aqueous phase was removed. Then 5 ml. of a solution of $NaHCO_3$ (0·4M) and Na_2CO_3 (0·4M) was added and the sample mixed as before. The ethyl acetate layer was discarded. The solution was heated to about 30° and re-extracted with 5 ml. of ethyl acetate. The heating prevented cloudiness from appearing later, which sometimes formed when the temperature of a solution previously cooled by evaporation into the air stream rose again to room temperature. The extinction at 367·5 mμ was read. The $E_{1\,cm.}$ of the hydrazone in the final solution was 3·85 if 1 μmole of pyruvoylglycine (see below) was present in the sample.

Previous methods have converted the yellow dinitrophenylhydrazone into a red form by adding alkali, but the red colour produced from the hydrazones of many oxo acids is unstable., The extinction of the red form which is maximal near 420 mμ is no higher than that at 367·5 mμ of the yellow and visually much paler compound than is present at a lower pH (Koepsell & Sharpe, 1952). A buffer of carbonate and bicarbonate was substituted for carbonate alone because the latter deepened the colour of the dinitrophenylhydrazone of glyoxylate to a reddish orange with a fall in the extinction at 367·5 mμ. This was presumably due to partial conversion into the red form at the higher pH.

Pyruvoyl-peptides and pyruvic acid may, alternatively, be detected by their own extinctions at 340 mμ (Errera & Greenstein, 1947), but this test is much less sensitive since a millimolar solution has an extinction of about 0·02 for a 1 cm. light-path.

Synthesis of pyruvoylglycine. Toluene-*p*-sulphonyl chloride (19·5 g., about 0·102 mole) was dissolved in acetone (50 ml.). Pyruvic acid (7·0 ml., 0·1 mole) was added and the solution cooled at 0°. Pyridine (8·1 ml., 0·1 mole) was added slowly with stirring and the solution allowed to stand at 0° for 20 min. A solution of glycine (7·5 g., 0·1 mole) and pyridine (17·0 ml., 0·21 mole) in 70 ml. of water was prepared and cooled at 0°. The solution in acetone was poured into the aqueous solution and stirred rapidly until a precipitate that formed had redissolved. During the mixing the temperature of the solution rose to 21°. After 20 min. the solution was reduced to a syrup by rotary evaporation, acidified with 10 ml. of 12N-HCl and made to 50 ml. with water. The solution was extracted four times with 200 ml. of ethyl acetate and the combined extract evaporated to dryness. The product was dissolved in 100 ml. of ether, shaken with 5 g. of Na_2SO_4, filtered and dried down. The final syrup was mixed with 20 ml. of N-NaOH and chromatographed as described above on a column with a resin bed of 60 cm. × 2 cm. Elution and detection of the products in the effluent were carried out as described above. Fig. 2 shows the pattern obtained. If the column is to be used again without regeneration with HCl, elution with the acetate buffer must be continued until material with high extinction at 270 mμ, which runs more slowly than pyruvic acid, is eluted, otherwise it is likely to contaminate sub-

sequent preparations. The fractions of effluent that contained the product were combined and passed through a column of sulphonated polystyrene resin (Zeo-Karb 225 SRC 13; H^+ form; 14–52 mesh; 8% cross-linking) and the effluent solution was dried by rotary evaporation. The syrup was dissolved in ether and dried again. After repeating this a few times the product crystallized. It was recrystallized from ethyl acetate by the addition of cyclohexane. The yield was 1·70 g. (12%). The yield calculated from the area of the peak was 13%. The compound had m.p. 89–90° (cf. 90° of Wieland, Shin & Heinke, 1958, and earlier workers) (Found: C, 41·35; H, 5·05; N, 9·6. $C_5H_7NO_4$ requires C, 41·38; H, 4·86; N, 9·65%).

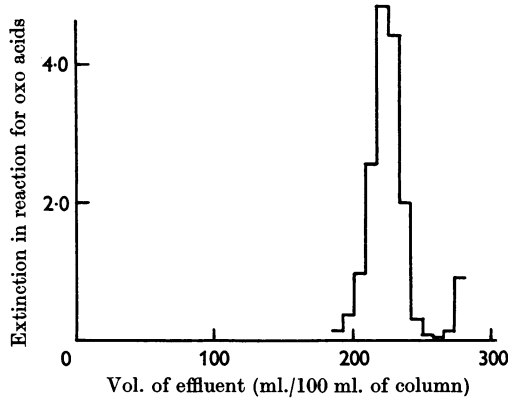


Fig. 2. Preparative chromatogram of pyruvoylglycine. A column (60 cm. × 2 cm.) was loaded with the crude product and developed as described in the text and in Fig. 1. The colour reaction was carried out on 0·1 ml. samples of 1:50 dilutions of the effluent.

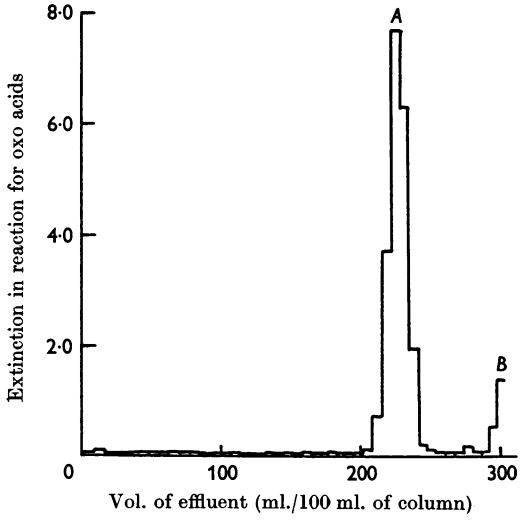


Fig. 3. Chromatogram of the product of transamination. The 10 ml. of reaction mixture from 1 m-mole of alanylglycine was applied directly to a column (60 cm. × 2 cm.) developed as described in the text and in Fig. 1. The colour reaction was carried out on 0·1 ml. samples of effluent. *A*, Pyruvoylglycine; *B*, start of glyoxylic acid.

Transamination of peptides. A solution was prepared of the peptide (0·1 M), glyoxylic acid (1·0 M), pyridine (10%, v/v) and cupric acetate. Different amounts of cupric acetate were used and it was added as a 0·25 M solution in 0·1 M-acetic acid. The order of addition may be important, as it is in cases where stable Schiff bases are formed (Nunez & Eichhorn, 1962). After 15 min. at 20° the solution was chromatographed. When 1 m-mole of DL-alanylglycine was used with a cupric acetate concentration of 0·1 M, and the 10 ml. of solution chromatographed on a column of about 60 cm. × 2 cm., pyruvoylglycine was isolated after chromatography (Fig. 3) in the way described above. The yield calculated from the area of the peak was 71% and after recrystallization was 55% (80 mg.). Its m.p., 88–89°, was not depressed on mixing with a sample prepared by the synthesis described from pyruvic acid and glycine (Found: C, 41·16; H, 4·95; N, 10·15. $C_5H_7NO_4$ requires C, 41·38; H, 4·86; N, 9·65%).

In experiments in which glyoxylamide was to be used, tartaramide (0·55 M in the final solution) and $NaIO_4$ (0·5 M) were first dissolved together. The iodate produced was precipitated by adding barium acetate (0·25 M), since otherwise it runs on chromatograms close to pyruvoylglycine and interferes with the dinitrophenylhydrazine test. Acetic acid (1·0 M) was added to maintain the pH. The precipitate was centrifuged off before chromatography and washed with the chromatographic eluent.

When the area of a peak was required as a measure of yield, and no isolation was to be attempted, a 0·1 m-mole sample of peptide was used, and a column of about 40 cm. × 1 cm.

RESULTS

The product obtained from alanylglycine on treatment with glyoxylate (Fig. 3) was an oxo acid as judged by the formation of a dinitrophenylhydrazone which was extracted from ethyl acetate by alkali but not by acid. It was characterized as the expected pyruvoylglycine by melting-point, elementary analysis, the dull-blue–green colour given with nitroprusside and ammonia (cf. Wieland *et al.* 1958) and by comparison with a synthetic sample made as described above; the comparison involved mixed melting-point and chromatographic examination. It thus appears that transamination occurred.

Much lower yields were obtained with alanylglycine if smaller quantities of cupric acetate were added. Many side reactions are known (cf. Metzler *et al.* 1954*a*) and these may remove Cu^{2+} ions by reduction or by the formation of inactive complexes. The colour of the solution changes from blue to green, and, if the concentration of cupric acetate is low, to olive green (except at the surface).

The time of reaction was chosen as 15 min. because an incubation of 70 min. gave a yield very slightly larger than one of 10 min., but there was a marked fall (40%) in the concentration of glyoxylate.

With glycylglycine the product of transamination has not been characterized except as a chromatographic peak. When glyoxylate was used (concn. of Cu^{2+} ions 0·1 M) the yield was only 19 % on the assumption that the maximum colour yield was the same as with alanylglycine. Since transamination is less with glycine than with other amino acids in terms of the position of equilibrium (Nakada & Weinhouse, 1953; Metzler *et al.* 1954*a*), it is possible that it may also be less with glycylpeptides than with other peptides. If this is the cause of the low yield, it suggests that peptide combination favours the amino form, since a tenfold excess of glyoxylate was used. This has been supported by a rise in the yield to 35 % (on the same assumption) when glyoxylate was replaced by glyoxylamide, i.e. when the amino acceptor was also in peptide combination. The values diverged even more when the Cu^{2+} ion concentration was diminished eightfold (to 12·5 mM); a better yield was still obtained with glyoxylamide (48 %) and worse with glyoxylate (9 %).

'An uncomplicated measurement of the equilibrium position of this transamination reaction cannot be made because of interfering side reactions' (Metzler *et al.* 1954*a*). It is therefore uncertain whether the differences in yield are related to the differences in equilibrium position or to the susceptibilities of either products or reagents to destruction.

DISCUSSION

The conclusion that transamination of peptides has occurred is based on a number of lines of evidence. A product has been isolated from the reaction mixture of peptide, glyoxylate, cupric salt and pyridine, and it possesses the properties of an oxo acid. When the peptide was alanylglycine, the product exhibited many of the properties of pyruvoylglycine. This conclusion is reinforced by its similarity with the product of a synthesis from pyruvic acid and glycine. Since, however, this product is no better characterized, these arguments only reinforce each other to a limited extent. Transamination is, however, the most likely reaction to produce an oxo acid under the conditions used.

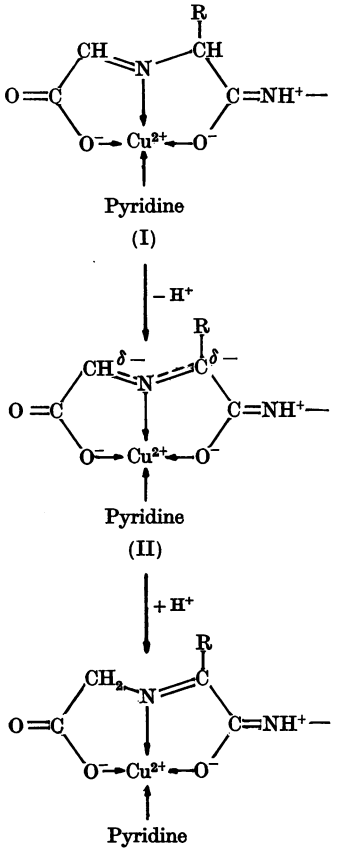
The synthesis made use of toluene-*p*-sulphonyl chloride for peptide synthesis, as suggested by various workers cited by Albertson (1962). Wieland *et al.* (1958) greatly simplified the synthesis of pyruvoylglycine. They prepared the benzyl ester of glycine, coupled this with pyruvic acid by using phosphorus oxychloride, and removed the protecting group by hydrogenation. The present method seems more convenient in spite of its lower yield (12 %), since a single step replaces three. Even if glycine benzyl ester is made by the convenient method of Cipera & Nicholls (1955), the procedure of Wieland *et al.* (1958) is long. Their synthesis needs care, since on three attempts the present author achieved nothing like the yield of 30 % claimed for the coupling step, probably because of insufficiently purified reagents and solvents. The present method has the disadvantage that chromatography is required to isolate the product. On one occasion pyruvoylglycine was crystallized without chromatography. The syrup of acids extracted by ethyl acetate was dissolved in aqueous sulphuric acid, potassium permanganate solution was added to oxidize free pyruvic acid until a colour persisted for a few seconds, the solution was adjusted to pH 2 and the acids remaining were again extracted into the ethyl acetate. The product was not satisfactory, probably because of contamination by the ultraviolet-absorbing material found on chromatography which might be *N*-toluene-*p*-sulphonylglycine.

The mechanism written by Mix (1961*a*) for Cu^{2+} ion-catalysed transamination, itself a slight modification of earlier proposals (cf. Metzler, Ikawa & Snell, 1954*b*), can be rewritten for the reaction between glyoxylate and a peptide as shown in Scheme 1. Here the Schiff base of the glyoxylate and peptide forms a complex with Cu^{2+} ions and pyridine (I). The electron-withdrawing properties of the Cu^{2+} ion assist the ionization to (II) and combination of a proton to the other side (possibly simultaneous with the formation of II) forms (III) and thus leads to transamination. The dissociation of protons from adjacent peptide bonds after initial binding of the terminal amino group by the Cu^{2+} ion (cf. Dobbie & Kermack, 1955; Martin, Chamberlin & Edsall, 1960; Koltun, Roth & Gurd, 1963; Campbell, Chu & Hubbard, 1963) need not be so complete as to preclude facilitation of the ionization of the α-hydrogen. The reaction has so far been demonstrated only with dipeptides where the dissociation of peptide protons will be least. The Schiff base may differ considerably from the peptide in its affinity for Cu^{2+} ions and in the way these are bound (cf. Nunez & Eichhorn, 1962).

The transamination has been carried out at room temperature (20°) and within the buffering range of pyridine (probably about pH 5·5). Cennamo *et al.* (1956) obtained transamination of peptides with pyridoxal as amino acceptor and alum as catalyst, but this method, like those previously used for amino acids, required high temperatures. The present method uses a low temperature, thanks to the great increase in the speed of the Cu^{2+} ion-catalysed reaction in the presence of pyridine (Mix & Wilcke, 1960; Mix, 1961*a*, *b*). Metzler & Snell (1952) preferred Al^{3+} ions to other ions as a catalyst because they could not act as an oxidizing agent. The possibility of accelerating the Cu^{2+} ion-

catalysed reaction with pyridine was the reason for rejecting this choice despite oxidative side reactions. Mild conditions have been sought in the hope that α-amino groups will react specifically without other change of proteins subjected to the procedure, but proteins have not yet been shown to transaminate specifically. Kurtz (1938), Campbell *et al.* (1963) and Collman & Buckingham (1963) similarly distinguished α-amino groups by combination with metal ions, McGregor & Carpenter (1962) also specifically converted the *N*-terminal residues of peptides into α-oxo acyl residues with hypobromite at pH 9·4, but the side chains of certain amino acids also reacted with the hypobromite. If the *N*-terminal residue is serine or threonine it is probably simpler to convert it into an oxo acyl residue by periodate oxidation than by transamination.

It is not easy to isolate glyoxylamide although its hydrate can be precipitated from acetic acid (Tits & Bruylants, 1948). It is therefore convenient to prepare it in the reaction mixture as described.



Scheme 1

It is known from a considerable body of work (cf. Nakada & Weinhouse, 1953; Metzler & Snell, 1952) that the position of equilibrium favours acceptance of an amino group by X–CO–CO–Y more when X = H than when X = alkyl, in cases where Y = OH. It is probable from the results described that this applies also when Y = NH–R and that the compound improves as an amino acceptor when Y is changed from OH to NH–R. This conclusion, which differs from that of Cennamo *et al.* (1956), depends, however, on the assumption that differences in yield with glyoxylate and glyoxylamide, and with glycyl- and alanyl-peptides, were due to differences in the position of equilibrium rather than to variations in the extent of side reactions.

The transamination may be of use in introducing an oxo group into a specific site in a peptide. The unique reactivity of this group may then allow further specific reactions. Dixon (1964) has described the use of thiosemicarbazide to remove the terminal residue after transamination. Preliminary experiments suggest that the oxo acyl residue may be removed after conversion into its selenosemicarbazone under conditions milder than those necessary for removal of its thiosemicarbazone. The selenosemicarbazone may be prepared by treatment with acetone selenosemicarbazone (Huls & Renson, 1956).

SUMMARY

1. Pyruvoylglycine has been prepared from alanylglycine by transamination at 20° by using a cupric salt and pyridine as catalysts. An oxo acid believed to be glyoxyloylglycine was similarly formed from glycylglycine.

2. Oxo acids have been separated by chromatography on basic resins.

3. A method of preparing pyruvoylglycine from pyruvic acid and glycine in 12 % yield by using toluene-*p*-sulphonyl chloride is described.

4. The possible use of transamination to allow specific removal of the *N*-terminal residue of a peptide is indicated.

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REFERENCES

Albertson, N. F. (1962). *Org. React.* **12**, 157.

Campbell, B. J., Chu, F. S. & Hubbard, S. (1963). *Biochemistry*, **2**, 764.

Cennamo, C., Carafoli, B. & Bonetti, E. P. (1956). *J. Amer. chem. Soc.* **78**, 3523.

Cipera, J. D. & Nicholls, R. V. V. (1955). *Chem. & Ind.* p. 16.

Collman, J. P. & Buckingham, D. A. (1963). *J. Amer. chem. Soc.* **85**, 3039.
Dixon, H. B. F. (1964). *Biochem. J.* **90**, 2c.
Dixon, H. B. F. & Weitkamp, L. R. (1962). *Biochem. J.* **84**, 462.
Dobbie, H. & Kermack, W. O. (1955). *Biochem. J.* **59**, 257.
Errera, M. & Greenstein, J. P. (1947). *Arch. Biochem.* **15**, 445.
Fleming, L. W. & Crosbie, G. W. (1960). *Biochim. biophys. Acta*, **43**, 139.
Friedemann, T. E. & Haugen, G. E. (1943). *J. biol. Chem.* **147**, 415.
Huls, R. & Renson, M. (1956). *Bull. Soc. chim. Belg.* **65**, 684.
Koepsell, H. J. & Sharpe, E. S. (1952). *Arch. Biochem. Biophys.* **38**, 443.
Koltun, W. L., Roth, R. H. & Gurd, F. R. N. (1963). *J. biol. Chem.* **238**, 124.
Kurtz, A. C. (1938). *J. biol. Chem.* **122**, 477.
Lu, G. D. (1939). *Biochem. J.* **33**, 249.
McGregor, W. H. & Carpenter, F. H. (1962). *Biochemistry*, **1**, 53.
Martin, R. B., Chamberlin, M. & Edsall, J. T. (1960). *J. Amer. chem. Soc.* **82**, 495.
Metzler, D. E., Ikawa, M. & Snell, E. E. (1954*b*). *J. Amer. chem. Soc.* **76**, 648.
Metzler, D. E., Olivard, J. & Snell, E. E. (1954*a*). *J. Amer. chem. Soc.* **76**, 644.
Metzler, D. E. & Snell, E. E. (1952). *J. Amer. chem. Soc.* **74**, 979.
Mix, H. (1961*a*). *Hoppe-Seyl. Z.* **323**, 173.
Mix, H. (1961*b*). *Hoppe-Seyl. Z.* **325**, 106.
Mix, H. & Wilcke, F. (1960). *Hoppe-Seyl. Z.* **318**, 148.
Nakada, H. I. & Weinhouse, S. (1953). *J. biol. Chem.* **204**, 831.
Nunez, L. J. & Eichhorn, G. L. (1962). *J. Amer. chem. Soc.* **84**, 901.
Tits, M. & Bruylants, A. (1948). *Bull. Soc. chim. Belg.* **57**, 50.
Wieland, T., Shin, K. H. & Heinke, B. (1958). *Chem. Ber.* **91**, 483.

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Sequences of Consecutive Cytosine Deoxynucleotides in Deoxyribonucleic Acid

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In previous work, up to seven consecutive thymine deoxynucleotides have been found in calf-thymus DNA (Burton & Petersen, 1960; Petersen, 1963*a*), but sequences of more than four consecutive cytosine deoxynucleotides have not been detected (Petersen, 1963*a*; Hall & Sinsheimer, 1963; Spencer & Chargaff, 1963). It was important to investigate the occurrence of pentadeoxycytidylate sequences because their absence would have strongly suggested the absence of CCC (and GGG) coding triplets (see Burton, Lunt, Petersen & Siebke, 1963). By using large samples of DNA we have been able to isolate and identify pentadeoxycytidylate sequences from the three deoxyribonucleic acids we have examined. Some of these results have already been briefly reported (Petersen, 1963*b*; Burton *et al.* 1963).

In the present paper we also include some observations on the degradation of DNA by aqueous formic acid at 30° and also by 0·1 M-sulphuric acid at 100°. The latter experiments were undertaken because tetradeoxycytidylate sequences had not been found by this method (Spencer & Chargaff, 1963).

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MATERIALS AND METHODS

Deoxyribonucleic acid. DNA was prepared from calf thymus by the method of Kay, Simmons & Dounce (1952), and from herring testis by the method of Emanuel & Chaikoff (1953). *Micrococcus lysodeikticus* DNA was prepared essentially by the method of Marmur (1961). The bacteria were grown at 30–35° with aeration in a medium containing 1% of tryptone, 0·5% of yeast extract, 0·5% of NaCl and 0·2% of glucose. Antifoam A (Hopkin and Williams Ltd.) was added as required. The cells (approx. 280 g. wet wt.) from 30 l. batches were suspended in 0·15 M-NaCl, pH 8 (1·5 ml./g. wet wt.). Lysozyme (0·7 mg./l.) was added and the cells were lysed at 37° with gentle stirring. To obtain satisfactory yields of DNA we found it necessary to add EDTA after lysis, 0·5 vol. of 0·3 M-EDTA, pH 8·0, containing NaCl (0·15 M) being used. The procedure of Marmur (1961) was then followed. The product contained much RNA, even after the ribonuclease treatment. Most of the RNA was removed by adsorption on to charcoal (Zamenhof & Chargaff, 1951). The final DNA was contaminated with non-nucleotide material, probably polysaccharide, and still contained 3·3% of the total phosphate as RNA P, as determined by the method of Webb (1956).

Ion-exchange resins. Dowex 1 (X8) and Dowex 50 (both 200–400 mesh) were washed as described by Burton & Petersen (1960). DEAE-Sephadex A-25, medium grade